

High Levels of α -Tocopherol in Norwegian Alpine Grazing Plants

Hanne Sickel*

Bioforsk – Norwegian Institute for Agricultural and Environmental Research, NO-2940 Heggnes, Norway

Wolfgang Bilger

Institute of Botany, Christian-Albrechts-University of Kiel, Olshausenstrasse 40, 24098 Kiel, Germany

Mikael Ohlson

Department of Ecology and Natural Resource Management, Norwegian University of Life Science (UMB), NO-1432 Ås, Norway

ABSTRACT: Antioxidants prevent oxidation of fatty acids in milk and meat. In the present study, the content of tocopherol antioxidants (vitamin E) in vegetative and reproductive parts of 22 grazing plants was estimated in two alpine areas used for summer farming. The overall mean content of α -tocopherol was $135 \pm 34 \mu\text{g g}^{-1}$ DW, and grasses had much lower content ($28 \pm 11 \mu\text{g g}^{-1}$ DW) than herbs ($215 \pm 94 \mu\text{g g}^{-1}$ DW), sedges ($186 \pm 78 \mu\text{g g}^{-1}$ DW), and woody species ($178 \pm 52 \mu\text{g g}^{-1}$ DW). Highest and lowest species-specific levels were 649 ± 91 and $2 \pm 1 \mu\text{g g}^{-1}$ DW, respectively. Plants from light and shady habitats did not differ in their α -tocopherol content, which was idiosyncratic as indicated by significant interactions between species, sampling occasion, site, and tissue type. Our results show that alpine ranges provide fodder with high levels of α -tocopherol.

KEYWORDS: *alpine ranges, seminatural grasslands, alpine plants, tocopherol, vitamin E*

■ INTRODUCTION

In Norway, mountain summer-farming is still practiced, and milk and meat are produced on species-rich alpine ranges where livestock graze on wild alpine plants. Milk and meat produced in the summer by animals that graze alpine ranges are expected to be of a unique quality as compared to production originating from lowland pastures.^{1–4} This may foster territorially anchored brands⁵ and create a competitive advantage in the market.⁶ Several studies the last 10 years have shown that such milk and meat have a healthier fatty acid composition than milk and meat produced on lowland pastures because of higher levels of polyunsaturated fatty acids (PUFA).^{7–10} However, higher levels of PUFAs may lead to reduced shelf life because the PUFAs render the products more susceptible to oxidation.¹¹ Lipid oxidation can give off flavors in milk¹¹ and meat products¹² as well as discoloration in meat.¹³

The levels of antioxidants such as carotenoids and tocopherols, also known as vitamin E, in milk and meat are considered to be of importance for the prevention of oxidation of PUFAs,^{14,15} and the concentration of these antioxidants in the animal products depends on the diet of the animal.^{16,17} α -Tocopherol (α -toc) is the major lipid soluble antioxidant in animal tissue¹⁵ and is also considered the most important antioxidant in milk. Pasture and grass silage give milk with higher concentrations of α -toc and carotenoids than hay and concentrates.¹⁸ Most studies on the levels of α -toc in pasture and silage have been done in the lowlands and on cultivated grass species. Interestingly, Leiber et al.⁸ found that pasture feeding increased milk α -toc concentration by 86% and 134% at low and high altitude, respectively, as compared to silage-concentrate diet. However, knowledge of the variation in α -toc

content between and within wild grazing plant species and sites on alpine ranges is very limited. Such knowledge may be of importance for developing production systems, grazing regimes, and management practices that secure high enough levels of antioxidants in the diet when utilizing alpine pastures or ranges. Today, alpine seminatural grasslands in Norway are in various degrees of regrowth and partly invaded by shrubs and trees that decrease the value of the grazing resources.¹⁹

Alpine plants are adapted to cope with severe stress factors such as low temperatures and high UV irradiance during a short growing season.²⁰ To prevent photoinhibition and to eliminate harmful reactive oxygen species that are formed under high UV irradiance, alpine plants are typically equipped with efficient enzymatic antioxidative systems and high levels of antioxidants.^{21,22} These antioxidants are generally water and lipid soluble, and their functional role has attracted considerable research over the last years as reviewed by, for example, Falk and Munné-Bosch.²³

Tocopherols, that is, α -toc, beta-tocopherol (β -toc), gamma-tocopherol (γ -toc), and delta-tocopherol (δ -toc), are a group of tocopherol derivatives that have a high antioxidant capacity.^{23,24} The levels of tocopherols have been found to be generally high in some alpine and arctic plants, and the levels tend to increase with altitude.^{21,22,25} These plants are thus assumed to have a tocopherol-based antioxidant system. Moreover, light conditions are known to impact on the levels

Received: April 24, 2012

Revised: July 7, 2012

Accepted: July 9, 2012

Published: July 9, 2012

Table 1. Climate Data for the Study Sites in Valdres and Hallingdal^a

climate stations and sampling dates in 2009	mean temp (°C)			temp (°C) at 8 a.m. and 2 p.m.	precipitation (mm)
	June	July	Aug		
Valdres Nord-Aurdal (639 m a.s.l.)					
1961–1990	10.7	12.1	11.0		604
2009	11.0	13.1	11.5		
sampling dates					
13 July				12.9, 15.0	0.0
14 July				11.3, 17.5	0.7
10 Aug				10.5, 15.6	8.0
11 Aug				10.1, 17.7	5.9
Hallingdal Hol (810 m a.s.l.)					
1961–1990	9.8	11.2	10.2		700
Hallingdal Geilo (772 m a.s.l.)					
2009	10.1	12.8	10.9		
sampling dates					
20 July				9.8, 10.7	1.5
21 July				10.7, 11.2	3.3
17 Aug				10.0, 13.8	0.5
18 Aug				7.8, 12.9	0.0

^aMean temperature for the months June, July, and August (normal and for 2009) and mean annual precipitation (normal) at the nearest weather stations to the study sites are given. Temperatures measured at 8 am and 2 pm and mean daily precipitation at the weather stations on the sampling dates are also reported. At the weather station in Valdres, Nord Aurdal, the average precipitation during the year amounts to 604 mm, mostly summer rain. The yearly precipitation in Hallingdal amounts to 700 mm. The study sites in Valdres and Hallingdal are located 270 m and 210/250 m, respectively, higher than the weather stations, and the temperatures at the study sites are therefore lower than the values given in the table. Temperature decreases approximately 0.6 °C per 100 m increase in elevation according to Körner 2003.²⁰ In Hallingdal, the weather station Hol was replaced by the weather station Geilo in 2006.

of tocopherols; plants from light exposed habitats typically have higher levels than plants from shady habitats.^{26,27} Levels of tocopherols increase with the age of the plant and may rise significantly at the end of the growth season.²⁶ In addition, different forms of tocopherols dominate in different types of plant tissue; that is, α -toc is generally most abundant in photosynthetic tissue such as leaf material, whereas γ -toc is characteristic of flowers and seeds.^{28–30}

Our main aim is to estimate tocopherol levels in alpine plants that are important as fodder for the grazing livestock in Norwegian summer farming areas. More specifically, we aim at identifying site- and species-specific patterns in tocopherol levels in 22 plant species that are abundant grazing plants in Norwegian seminatural grasslands.^{19,31,32} We also compare the tocopherol levels between leaves and flowers and between shady and exposed habitats. Finally, we compare mid- and late-summer levels of tocopherol.

MATERIALS AND METHODS

Study Sites and Plant Samples. Our study sites are seminatural grasslands in the alpine regions Valdres and Hallingdal of south-central Norway. The study site in Valdres (60°57' N; 8°49' E) is situated approximately 910 m a.s.l., in the northern boreal vegetation zone.³³ The bedrock in the area is phyllite, a schist-rich bedrock. This bedrock has a high weathering capacity, thus giving rise to soils of intermediate or good nutritional quality for plants. The study site in the Hallingdal region (60°32' N; 8°11' E) is located approximately 1040 m a.s.l. in the transition between the northern boreal and low alpine vegetation zone.³³ The bedrock in the area is mainly metarhyolite and metarhyodacite, which has low weathering capacity and low nutritional quality for plants. However, parts of the area are influenced by metatuffites or by seepage water from neighboring areas with phyllite, which improves the nutrient status of the soils. Climate data from the nearest weather stations are given in Table 1.

Both study sites are traditional summer farming areas where dairy cattle, and other domestic animals (sheep, horses, goats), have grazed

for centuries. Mountain birch forests of maximum 5–6 m height are present in both our study areas.

Twenty-two plant species (Table 2) were collected in seminatural grasslands from the sites in July and August 2009 (except for *Poa pratensis*, which was found only in cultivated pastures). The samples were collected in two habitat types, light exposed open and shady forested regrowth habitats. All sampling took place between 0900 and 1700 h because diurnal changes in tocopherol levels are small during this period of the day.²¹ Vegetative tissue (leaves) was sampled from all species and mostly from lower parts of the plants because many of them (especially the grasses and the herbs) have their leaves based near the ground. Reproductive tissue (flowers or bulbils) was sampled from a subset of four species. Detailed information about habitats, sampling dates, sampled plant species, tissue type, and number of replicates is shown in Tables 1 and 2.

Analysis. The samples were transported to the laboratory in liquid nitrogen and thereafter stored at –80 °C. The samples were then freeze-dried within 2 months, stored at –20 °C, and analyzed for tocopherols at the University of Kiel, Department of Botany. The analysis procedure for tocopherols was as follows.

Freeze-dried material was ground using a mill (Type A10, IKA-Werke, Staufen, Germany) for 15 s. After being ground, most particles were less than 400 μm^2 . The larger particles were fibers, which have a high surface to volume ratio favorable for extraction. Approximately 0.04 g of the ground material was put into microcentrifuge caps together with 1000 or 500 μL of *n*-heptane (Rotisol HPLC grade, Roth, Karlsruhe, Germany) and five steel balls (2 \times 4 mm and 3 \times 3 mm). After homogenization in a Geno Grinder (Type 2000, SPEX, CertiPrep, Munich, Germany) (3 min with 1100 strokes), more *n*-heptane was added to a final volume of 1500 or 2000 μL for leaf/mixed material or 1000 μL for pure flower material. The samples were processed using chilled equipment to prevent the overheating of the samples. The microcentrifuge caps were shortly shaken and stored overnight at –20 °C for extraction. After extraction, the caps were centrifuged for 7 min at 11 000g at 4 °C. 50 μL of the supernatant was transferred to microvials to be analyzed for tocopherols.

The extracts were analyzed according to Falk et al.³⁴ using a Shimadzu VP series HPLC system (Shimadzu, Duisburg, Germany)

Table 2. Species Sampled at the Sites in Valdres (V) and Hallingdal (H)^a

species	life form	short name	N	site	tissue type	habitat type	month
<i>Astragalus alpinus</i>	h	Aa	20	H	l	L, S	7, 8
<i>Bistorta vivipara</i>	h	Bv	20	V	l, r ^b	L, S	7, 8
<i>Leontodon autumnalis</i>	h	La	40	V, H	l, r	L, S	7, 8
<i>Parnassia palustris</i>	h	Pap	20	H	l, c, r	L, S	7, 8
<i>Trifolium repens</i>	h	Tr	30	V, H	l, r	L	7, 8
<i>Viola biflora</i>	h	Vb	5	H	l	L	7
<i>Carex bigelowii</i>	s	Cb	5	H	l	L	7
<i>Carex nigra</i>	s	Cn	10	V, H	l	L	7
<i>Carex vaginata</i>	s	Cv	5	V	l	L	7
<i>Juncus filiformis</i>	s	Jf	5	V	l	L	7
<i>Salix glauca</i>	w	Sg	10	V	l	L, S	7
<i>Salix herbacea</i>	w	Sh	5	H	l	L	7
<i>Salix phylicifolia</i>	w	Sp	5	H	l	L	7
<i>Vaccinium myrtillus</i>	w	Vm	10	V	l	L, S	7
<i>Agrostis capillaris</i>	g	Ag	20	V, H	l	L, S	7
<i>Anthoxanthum odoratum</i>	g	Ao	10	V	l	L, S	7
<i>Avenella flexuosa</i>	g	Af	20	V, H	l	L, S	7
<i>Deschampsia cespitosa</i>	g	Dc	5	V	l	L	7
<i>Festuca rubra</i>	g	Fr	8	V	l	L, S	7
<i>Phleum alpinum</i>	g	Pa	5	H	l	L	7
<i>Poa alpina</i>	g	Poa	10	H	l	L	7, 8
<i>Poa pratensis</i>	g	Pp	5	V	l	L	7

^aThere are five replicates for each combination of plant species, site, tissue type, habitat type, and sampling date (except for *Festuca rubra* where two samples were rejected due to sampling failure), giving a total of 273 samples. Life form: h = herbs, s = sedges, w = woody species, and g = grasses. Tissue type: l = leaf, r = reproductive. Exposure: L = light, S = shady. Month: 7 = July, 8 = August. Nomenclature for plant names follows.⁴³ ^bFlowers and bulbils. ^cFlower stalks.

equipped with a fluorescence detector (RF-10A XL, Shimadzu) and run by Class VP 7.4 SP1 software (Shimadzu). Tocopherols were separated isocratically on a LiChrosphere Si 60 column (5 μ m/250–4 mm, Merck, Darmstadt, Germany) using *n*-heptane/isopropanol (99:1 v/v) as an eluent. Twenty microliters of the samples was injected. The flow rate was 1 mL per minute. The fluorescence detector was calibrated with α -, β -, γ -, and δ -toc standards (Merck). Fluorescence of all components was excited at 290 nm and detected at 328 nm.

Statistical Analyses. Values are expressed as mean \pm 1 SE. Because of the unbalanced sampling design (Table 2), we used a set of four two-way analyses of variance (ANOVA) for comparisons among plant species, habitats, sampling occasions, sites, and tissue types. The confidence coefficient was set at 0.01 (i.e., a restrictive Bonferroni correction) to counteract the problem of multiple comparisons among means. Paired-sample *t* tests were used to compare vegetative and reproductive tissue as regarding their content of β -, γ -, and δ -toc, respectively. The analyses were performed by the software Minitab 16. To find the best fit to the normal distribution, the software suggested a transformation of the data by $1/y^5$, which was chosen and used in all cases.

To elucidate general differences in tocopherol contents among life forms of plants, the 22 study species were arranged in four groups according to their life form, that is, grasses (eight species), sedges (four species), herbs (six species), and woody species (four species); see Table 2.

RESULTS AND DISCUSSION

Content of Tocopherols. The tocopherol contents of the plant species varied considerably. Highest and lowest species-specific levels were 664 and 2 μ g g⁻¹ DW, respectively, and the overall mean content of tocopherols for the 22 plant species was 141 \pm 34 μ g g⁻¹ DW (Table 3). The lowest levels were found in the grass *Avenella flexuosa*, and grasses as a group had a tocopherol content that was about 4 times lower than that in herbs, sedges, and woody species (Figure 1).

The striking variation in α -toc content among the investigated plant species (Table 3) is in line with results from studies of other plant species in other ecosystems^{35,36} and supports the view that vascular plants are in general highly variable in their content of α -toc. Interestingly, some of the individual measurements for our plant species are at the high end of the scale of variation, that is, values up to 1091 and 849 μ g g⁻¹ DW in the herbs *L. autumnalis* and *Viola biflora*, respectively. These values match the highest levels in the studies of Wildi and Lütz²¹ and Lütz and Holzinger,²⁵ who measured the content of α -toc in selected alpine and arctic plants and found the highest levels in the herbs *Dryas octopetala*, *B. vivipara*, and *Soldanella pusilla*. The overall average content of 135 μ g g⁻¹ DW α -toc in our study plants is also relatively high as compared to corresponding figures for European lowland pasture plants and some tropical plants (Table 6). Consequently, we suggest that alpine grazing plants are characterized by generally high levels of α -toc. In particular, this is the case for herbs, sedges, and woody plants as indicated by an average α -toc level of 196 μ g g⁻¹ DW for the 14 species representing these three life forms. In contrast, the average level of 28 μ g g⁻¹ DW α -toc for the eight grass species in our study was relatively low. Here, it is noteworthy that the small-sized and fine-leaved grass species (i.e., *Poa alpina*, *Phleum alpinum*, *Anthoxanthum odoratum*, *Agrostis capillaris*, and *Avenella flexuosa*) had very low content of α -toc (2–16 μ g g⁻¹ DW), whereas the content for large and broad-leaved grasses (i.e., *Deschampsia cespitosa*, *Festuca rubra*, and *P. pratensis*) was significantly higher (48–82 μ g g⁻¹ DW). Our values for the large and broad-leaved grasses correspond well with reported values for other grass species, for example, *Lolium perenne*, cultivated in lowland pastures and used as forage for cattle (Table 6). However, the low α -toc content in the small-sized grasses was unexpected and may indicate that their antioxidant systems are not based on α -toc or, alternatively, that these species are not exposed to environmental stress factors that trigger an efficient antioxidant system. This is plausible simply because these grasses are small and are typically overgrown and shaded by other plants in our study sites, which in turn implies that the climatic stress level was relatively low in the overgrown plants.

Composition of Tocopherols. In all species, the tocopherol pool was dominated by α -toc, which made up 83–100% of the total pool. In general, β -toc and γ -toc were found only as small fractions of the total tocopherol pool, and δ -toc was practically absent in all plants. However, γ -toc made up a sizable part of the tocopherol pool in the dwarf shrub *Vaccinium myrtillus* (14%, Table 3). Reproductive tissue (flowers and bulbils) had significantly higher levels of β -, γ -, and δ -toc than vegetative tissue ($P \leq 0,001$), and, interestingly, *Bistorta vivipara* had equal amounts of γ - and α -toc in its reproductive tissue (Table 4).

Table 3. Content of Different Tocopherol Forms Measured in Norwegian Alpine Meadow Plants^a

plant species	α -toc ($\mu\text{g/g DW}$)	β -toc ($\mu\text{g/g DW}$)	γ -toc ($\mu\text{g/g DW}$)	δ -toc ($\mu\text{g/g DW}$)	total toc ($\mu\text{g/g DW}$)	flowers (%)	α -toc (%)
<i>Astragalus alpinus</i>	63 ± 10	1 ± 0	0 ± 0	nd	65 ± 10		97
<i>Bistorta vivipara</i>	186 ± 45	1 ± 0	13 ± 2	0 ± 0	200 ± 46	6 ± 1	93
<i>Leontodon autumnalis</i>	272 ± 45	4 ± 0	7 ± 1	0 ± 0	282 ± 46	25 ± 1	96
<i>Parnassia palustris</i>	88 ± 6	3 ± 0	3 ± 0	0 ± 0	94 ± 6	48 ± 4	94
<i>Trifolium repens</i>	33 ± 4	1 ± 0	2 ± 0	0 ± 0	36 ± 4	22 ± 1	92
<i>Viola biflora</i>	649 ± 91	4 ± 0	10 ± 1	0 ± 0	664 ± 92		98
<i>Carex bigelowii</i>	63 ± 11	1 ± 0	1 ± 1	nd	65 ± 11		97
<i>Carex nigra</i>	75 ± 9	1 ± 0	7 ± 4	nd	83 ± 10		90
<i>Carex vaginata</i>	399 ± 35	2 ± 0	2 ± 0	nd	404 ± 35		99
<i>Juncus filiformis</i>	208 ± 23	1 ± 0	4 ± 0	nd	214 ± 24		97
<i>Salix glauca</i>	48 ± 8	1 ± 0	3 ± 0	nd	52 ± 8		92
<i>Salix herbacea</i>	283 ± 26	3 ± 0	5 ± 1	0 ± 0	292 ± 26		97
<i>Salix phylicifolia</i>	234 ± 16	2 ± 0	7 ± 0	nd	242 ± 17		97
<i>Vaccinium myrtillus</i>	145 ± 21	1 ± 0	24 ± 3	0 ± 0	170 ± 23		85
<i>Agrostis capillaris</i>	3 ± 1	0 ± 0	0 ± 0	nd	3 ± 1		100
<i>Anthoxanthum odoratum</i>	16 ± 7	0 ± 0	0 ± 0	nd	16 ± 7		100
<i>Avenella flexuosa</i>	2 ± 1	0 ± 0	0 ± 0	nd	2 ± 1		100
<i>Deschampsia cespitosa</i>	82 ± 24	1 ± 0	1 ± 0	nd	84 ± 25		98
<i>Festuca rubra</i>	65 ± 6	0 ± 0	1 ± 0	nd	66 ± 6		98
<i>Phleum alpinum</i>	6 ± 1	nd	0 ± 0	nd	6 ± 1		100
<i>Poa alpina</i>	5 ± 2	0 ± 0	1 ± 0	nd	6 ± 2		83
<i>Poa pratensis</i>	48 ± 8	1 ± 0	1 ± 0	nd	50 ± 8		96

^aValues are means ± 1 SE. For samples that contain flowers, the weight fractions (%) of the flowers are given (mean values ± 1 SE).

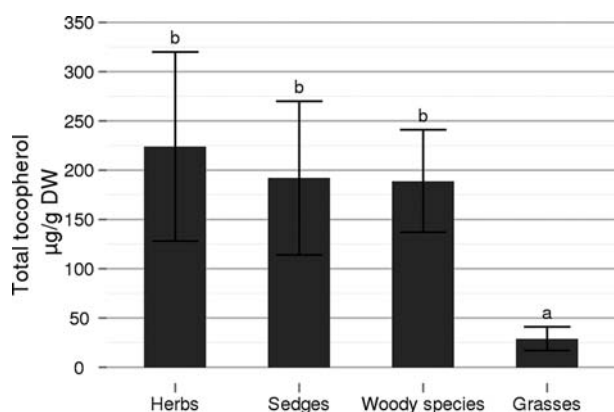


Figure 1. Content of tocopherol in herbs (six species), sedges (four species), woody species (four species), and grass species (eight species) sampled in alpine seminatural grasslands. Values are mean ± 1 SE, and different letters indicate significantly different values at the 0.05 level (ANOVA).

The tocopherol pool in leaves and other green parts of plants is known to be dominated by α -toc, which usually makes up >90% of the total tocopherol pool in such plant parts. Thus, as the plant material in our study consisted mainly of leaves, it was expected that the tocopherol pool would be dominated by α -toc, and our results correspond well with previous studies.²⁹

The γ -toc form is known to be a precursor of α -toc³⁷ and an indicator of the final stage of senescence and of stress-induced changes.³⁴ In addition, γ -toc may also play an important role as antioxidant in tissue that exhibits desiccation stress.^{23,38} It is thus plausible that the only species in our study that was characterized by a significant proportion of γ -toc in its tocopherol pool (*Vaccinium myrtillus*) experienced relatively high levels of stress. We think that this is reasonable as *V. myrtillus* is not an alpine plant and may struggle with the environmental conditions in alpine seminatural grasslands.

Table 4. Tocopherol Contents in Different Tissues of Norwegian Alpine Meadow Plants^a

species	α -toc ($\mu\text{g/g DW}$)	β -toc ($\mu\text{g/g DW}$)	γ -toc ($\mu\text{g/g DW}$)	δ -toc ($\mu\text{g/g DW}$)
Leaf/Flower Stalks				
<i>Bistorta vivipara</i> (n = 5)	99 ± 21	1 ± 0	13 ± 3	nd
<i>Leontodon autumnalis</i> (n = 25)	102 ± 13	2 ± 0	3 ± 0	nd
<i>Trifolium repens</i> (n = 15)	46 ± 7	1 ± 0	2 ± 0	nd
<i>Parnassia palustris</i> (n = 10)	81 ± 5	3 ± 0	2 ± 0	nd
Flower				
<i>Bistorta vivipara</i> (n = 5)	43 ± 14	1 ± 0	44 ± 11	5 ± 1
<i>Leontodon autumnalis</i> (n = 25)	158 ± 16	7 ± 2	14 ± 2	1 ± 0
<i>Trifolium repens</i> (n = 15)	9 ± 1	0 ± 0	6 ± 1	0 ± 0
<i>Parnassia palustris</i> (n = 10)	136 ± 7	4 ± 0	6 ± 0	0 ± 0

^aThe given values are mean ± 1 SE.

Seeds are generally characterized by a high content of γ -toc,²⁸ and the reproductive structures will be expected to contain gradually more γ -toc as seeds develop and mature during the end of the growing season. That there were about equal proportions of γ -toc and α -toc in reproductive tissue of *B. viviparum* (Table 4) is likely due to occurrence of bulbils (vegetative dispersal units) in the lower part of the flowering spike of this species. These bulbils may have been included in the analysis of the reproductive tissue for this species, and we think that this can explain the high proportion of γ -toc in the samples of this species. Bulbils of *Bistorta* are known to be

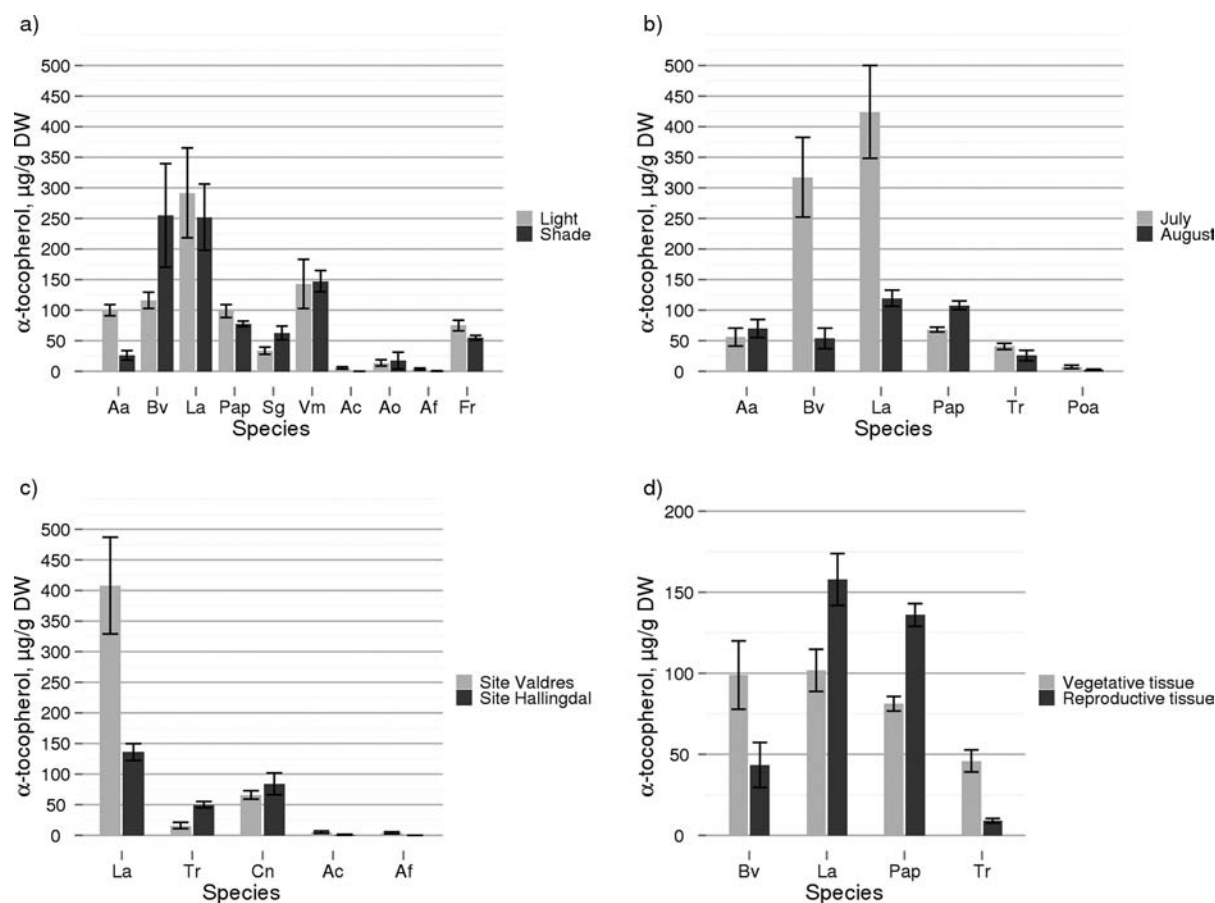


Figure 2. Content of α -tocopherol in Norwegian alpine grazing plants: (a) in light and shady habitats; (b) in July and August; (c) at two geographically different sites; and (d) in vegetative and reproductive tissue. Species orders along the horizontal axes are the same as in Table 2, which also gives the key to the abbreviated plant names. Values are mean \pm 1 SE.

nutritious and have been eaten both by animals and by humans in areas where the plant is abundant.^{39,40}

High proportions of α -toc were found in the flowers of *L. autumnalis* and *P. palustris*, which can probably be explained by high proportions of green tissue in their flowers (involucral bracts in several layers in *L. autumnalis* and green sepals of nearly the same size as the petals and green stamens in *P. palustris*) as green flower buds have been reported to be characterized by high α -toc proportions.³⁰

Variation Related to Habitats and Study Sites. ANOVA was used to search for patterns in the α -toc contents and revealed four main patterns. First, the content of α -toc is clearly species-specific; second, the average content of α -toc in plants from light and shady habitats did not differ; third, the average midsummer (July) level of α -toc was higher than the late summer (August) level; and fourth, a good deal of the variation in the α -toc content was idiosyncratic as indicated by significant interactions between species, sampling occasion, site, and tissue type (Figure 2 and Table 5). For example, *B. vivipara* and *L. autumnalis* had a higher content of α -toc in July than in August, whereas the content was highest in August for *Astragalus alpinus* and *Parnassia palustris* (Figure 2b). Moreover, *Trifolium repens* had about a 3 times higher content of α -toc at the Hallingdal site as compared to the Valdres site (50 versus 16 $\mu\text{g/g DW}$), while the opposite was the case for *L. autumnalis*, which had a much higher content at the Valdres site than at the Hallingdal site (408 versus 136 $\mu\text{g/g DW}$, respectively) (Figure 2c). Finally, reproductive tissue had a higher content of α -toc

Table 5. Summary of Analysis of Variance (ANOVA) on α -Tocopherol Levels in Norwegian Alpine Grazing Plants^a

	F	P
a (n = 178)		
species	26.21	<0.001
habitat	0.62	0.433
interaction	1.35	0.216
b (n = 140)		
species	33.99	<0.001
sampling occasion	13.78	<0.001
interaction	10.23	<0.001
c (n = 110)		
species	15.00	0.011
site	0.02	0.907
interaction	4.51	0.002
d (n = 110)		
species	37.87	<0.001
tissue type	0.20	0.658
interaction	11.15	<0.001

^a(a) In 10 species from two habitats (light and shady); (b) in six species at two sampling occasions (July and August); (c) in five species at two sites (Valdres and Hallingdal); and (d) in four species and two tissue types (vegetative and reproductive).

than vegetative tissue in *L. autumnalis* and *P. palustris*, but for *B. vivipara* and *T. repens* the content was highest in vegetative tissue (Figure 2d).

Table 6. Content of α -Tocopherol in Alpine/Arctic Plants from Other Studies and in Bulk Samples of Pasture and Silage from Lowland Studies^a

comparing values from other studies on alpine species (leaf samples)	α -toc ($\mu\text{g g}^{-1}$ FW)	ref	comparing values from other studies in the lowlands	α -toc ($\mu\text{g g}^{-1}$ DW)	ref
<i>Dryas octopetala</i> (a)	176	25	Brassicaceae/Graminae/Geraniaceae (d)	15–46	44
<i>Bistorta vivipara</i> (b)	110	25	grass-clover silage just after harvest (e)	32	45
<i>Soldanella pusilla</i> (c)	67	21	whole crop silage just after harvest (e)	51	45
nine alpine species (c)	68	21	grass-clover silage (f)	62	46
			whole crop silage (f)	17	46
			<i>Lolium perenne</i> silage (g)	63	47
			pasture of <i>L. perenne</i> , <i>T. repens</i> , <i>Poa</i> sp. (g)	23	47
			<i>Lolium multiflorum</i> (h)	4	48
			pasture grass (i)	14	49
			meadow grass (<i>Bromus</i> spp., <i>Holcus</i> spp., <i>Dactylis glomerata</i>) (i)	8	49
			silage, first cut (i)	2–31	49
			<i>Lolium perenne</i> , fresh (j)	156	50
			<i>Lolium perenne</i> , silage (j)	102	50
			62 species of edible tropical plants (k)	106	35

^aStudy sites: a = Germany, 2200 m a.s.l., b = Spitzbergen, c = Austria, 1000–3000 m a.s.l., d = Iberian Peninsula, e = organic farms, Denmark, f = conventional farms, Denmark, g = Ireland, h = Switzerland, 400 m a.s.l., i = Ireland, j = Belgium, and k = Malaysia.

As earlier studies have found higher levels of α -toc in sun-exposed plants than in plants in the shade,^{26,27} and that α -toc levels increase with plant age until peaking late in the season when the plants are in the early stages of senescence,²⁶ we had expected to find a clear pattern with higher levels of α -toc in the material originating from light-exposed open habitats and from the last sampling in August. However, this was not the case. On the contrary, we found no difference between light and shady habitats, and one-half the number of study species had their highest levels of α -toc at the first sampling in July (see Figure 2). This suggests that the variation in α -toc levels is individualistic and not related to the light conditions of the habitat or the time of the season in a straightforward and systematic way. That we found no consistent increase in α -toc levels over the season may be due to the life-form of the study species. Our data are for grass and herbaceous species, while the data to which we compare our results are for woody species²⁶ in which the main role for photoprotective compounds is to enhance the nitrogen remobilization and storage at the end of the season. One reason for the unexpected lack of a systematic difference between light and shady habitats may lie in the particular light conditions of the plants sampled in the open and reforested habitats. They may not have been significantly different because the plants (except *P. pratensis*) were more or less shaded by taller growing herbs, also in the open seminatural grassland. These taller herbs consisted of species not grazed by cattle, for example, *Aconitum septentrionale* and *Geranium sylvaticum*. In situ measurements of the light conditions are needed to clarify this. Also, the woods, consisting mainly of *B. pubescens*, only cast partial shadow on the plants, thus still allowing growth of plants adapted to comparatively high light exposure.

It has been shown that the content of α -toc in plants is affected by geographic location,³⁰ and in our study two of the species (i.e., *L. autumnalis* and *T. repens*) had significantly different contents of α -toc between the two geographically different sampling sites (the significant interaction between species and site in Table 5). We do not know the reason for this variation, but differences in microenvironmental conditions that are site-specific are likely an important part of the explanation for the difference between the sites regarding these two species. Such idiosyncratic variation seems to be typical for

higher plants as also the mineral concentration levels in wild grazing plants are known to be significantly site- and species-specific.⁴¹ However, a genetic component may also contribute significantly to the variation, because the content of tocopherol is known to differ between varieties of barley.⁴² Carefully designed experiments would help reveal if it is alpine genotypes or the alpine environment that is the main driver of high levels of tocopherol or other antioxidants in alpine plants.

High Content of α -toc in Alpine Grazing Plants. We conclude that high levels of α -toc were measured in several wild growing grazing plants in the seminatural grasslands of two alpine regions in Norway. These alpine grazing plants seem to be significantly richer in vitamin E as compared to cultivated pasture plants in the lowland. Our results are an argument for continuing the practice of cattle grazing in alpine seminatural grasslands. A high fraction of herbs, sedges, and woody species as compared to grasses is the main reason for the high α -toc content.

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: +4740483467. Fax: +4761340665. E-mail: hanne.sickel@bioforsk.no.

Funding

This work was supported by The Research Council of Norway.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are very grateful to Jens Hermann at the Institute of Botany, Christian-Albrechts-University of Kiel, for technical assistance. We also thank Kristina Norderup, Ulla Falkdalen, Kristin Daugstad, and Jørgen Todnem at Bioforsk Løken for their assistance during the field work. We are grateful to the Norwegian Meteorological Institute and Norwegian Geological Surveys for supplying us with climate data and bedrock data.

■ ABBREVIATIONS USED

PUFA, polyunsaturated fatty acid; DW, dry weight; FW, fresh weight; α -toc, alpha-tocopherol; β -toc, beta-tocopherol; γ -toc, gamma-tocopherol; δ -toc, delta-tocopherol.

REFERENCES

- (1) Mariaca, R. G.; Berger, T. F. H.; Gauch, R.; Imhof, M. I.; Jeangros, B.; Bosset, J. O. Occurrence of volatile mono- and sesquiterpenoids in highland and lowland plant species as possible precursors for flavor compounds in milk and dairy products. *J. Agric. Food Chem.* **1997**, *45*, 4423–4434.
- (2) Prache, S.; Cornu, A.; Berdague, J. L.; Priolo, A. Traceability of animal feeding diet in the meat and milk of small ruminants. *Small Ruminant Res.* **2005**, *59*, 157–168.
- (3) Lucas, A.; Agabriel, C.; Martin, B.; Ferlay, A.; Verdier-Metz, I.; Coulon, J. B.; Rock, E. Relationships between the conditions of cow's milk production and the contents of components of nutritional interest in raw milk farmhouse cheese. *Lait* **2006**, *86*, 177–202.
- (4) Schjøll, A.; Amilien, V.; P.A., T.; Revoredo-Giha, C.; Leat, P.; Kupiec, B.; Lamprinopoulou, C. Promotion of mountain food: An explorative study about consumers' and retailers' perception in six European countries. In *Building Sustainable Rural Futures. The Added Value of Systems Approaches in Times of Change and Uncertainty. 9th European IFSA Symposium, Vienna, Austria, July 4–7, 2010*; Darnhofer, I., Grötzer, M., Eds.; Universität für Bodenkultur: Vienna, Austria, 2010; pp 1558–1567.
- (5) Povolito, M.; Pelizzola, V.; Lombardi, G.; Tava, A.; Contarini, G. Hydrocarbon and fatty acid composition of cheese as affected by the pasture vegetation type. *J. Agric. Food Chem.* **2012**, *60*, 299–308.
- (6) Rytönen, P.; Grätzer, K. When do trademarks create new markets? Entrepreneurship, brands and growth – experiences from small scale cheese production in Austria, Spain and Sweden. In *Building Sustainable Rural Futures. The Added Value of Systems Approaches in Times of Change and Uncertainty. 9th European IFSA Symposium, Vienna, Austria, July 4–7, 2010*; Darnhofer, I., Grötzer, M., Eds.; Universität für Bodenkultur: Vienna, Austria, 2010; pp 1661–1670.
- (7) Collomb, M.; Butikofer, U.; Sieber, R.; Jeangros, B.; Bosset, J. O. Composition of fatty acids in cow's milk fat produced in the lowlands, mountains and highlands of Switzerland using high-resolution gas chromatography. *Int. Dairy J.* **2002**, *12*, 649–659.
- (8) Leiber, F.; Kreuzer, M.; Nigg, D.; Wettstein, H. R.; Scheeder, M. R. A study on the causes for the elevated n-3 fatty acids in cows' milk of alpine origin. *Lipids* **2005**, *40*, 191–202.
- (9) Ádnøy, T.; Haug, A.; Sørheim, O.; Thomassen, M. S.; Varszegi, Z.; Eik, L. O. Grazing on mountain pastures – does it affect meat quality in lambs? *Livest. Prod. Sci.* **2005**, *94*, 25–31.
- (10) Lind, V.; Berg, J.; Eik, L. O.; Møllmann, J.; Haugland, E.; Jørgensen, M.; Hersleth, M. Meat quality of lamb: Pre-slaughter fattening on cultivated or mountain range pastures. *Meat Sci.* **2009**, *83*, 706–712.
- (11) Charmley, E.; Nicholson, J. W. G. Influence of dietary-fat source on oxidative stability and fatty-acid composition of milk from cows receiving a low or high level of dietary vitamin-E. *Can. J. Anim. Sci.* **1994**, *74*, 657–664.
- (12) Nute, G. R.; Richardson, R. I.; Wood, J. D.; Hughes, S. I.; Wilkinson, R. G.; Cooper, S. L.; Sinclair, L. A. Effect of dietary oil source on the flavour and the colour and lipid stability of lamb meat. *Meat Sci.* **2007**, *77*, 547–555.
- (13) Luciano, G.; Monahan, F. J.; Vasta, V.; Pennisi, P.; Bella, M.; Priolo, A. Lipid and colour stability of meat from lambs fed fresh herbage or concentrate. *Meat Sci.* **2009**, *82*, 193–199.
- (14) Havemose, M. S.; Weisbjerg, M. R.; Bredie, W. L. P.; Nielsen, J. H. Influence of feeding different types of roughage on the oxidative stability of milk. *Int. Dairy J.* **2004**, *14*, 563–570.
- (15) Wood, J. D.; Enser, M. Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *Br. J. Nutr.* **1997**, *78*, 49–60.
- (16) Calderon, F.; Chauveau-Duriot, B.; Pradel, P.; Martin, B.; Graulet, B.; Doreau, M.; Nozière, P. Variations in carotenoids, vitamins A and E, and color in cow's plasma and milk following a shift from hay diet to diets containing increasing levels of Carotenoids and vitamin E. *J. Dairy Sci.* **2007**, *90*, 5651–5664.
- (17) Prache, S. Diet authentication in sheep from the composition of animal tissues and products. *Rev. Bras. Zootec.* **2009**, *38*, 362–370.
- (18) Martin, B.; Hurtaud, C.; Graulet, B.; Ferlay, A.; Chilliard, Y.; Coulon, J. B. Grass and the nutritional and organoleptic qualities of dairy products. *Fourrages* **2009**, 291–310.
- (19) Sæther, N. H.; Sickel, H.; Norderhaug, A.; Sickel, M.; Vangen, O. Plant and vegetation preferences for a high and a moderate yielding Norwegian dairy cattle breed grazing semi-natural mountain pastures. *Anim. Res.* **2006**, *55*, 367–387.
- (20) Körner, C. *Alpine Plant Life. Functional Plant Ecology of High Mountain Ecosystems*, 2nd ed.; Springer: Heidelberg, Germany, 2003; p 344.
- (21) Wildi, B.; Lutz, C. Antioxidant composition of selected high alpine plant species from different altitudes. *Plant Cell Environ.* **1996**, *19*, 138–146.
- (22) Streb, P.; Shang, W.; Feierabend, J.; Bligny, R. Divergent strategies of photoprotection in high-mountain plants. *Planta* **1998**, *207*, 313–324.
- (23) Falk, J.; Munne-Bosch, S. Tocochromanol functions in plants: antioxidation and beyond. *J. Exp. Bot.* **2010**, *61*, 1549–1566.
- (24) Asensi-Fabado, M. A.; Munne-Bosch, S. Vitamins in plants: occurrence, biosynthesis and antioxidant function. *Trends Plant Sci.* **2010**, *15*, 582–592.
- (25) Lütz, C.; Holzinger, A. A comparative analysis of photosynthetic pigments and tocopherol of some arctic-alpine plants from Kongsfjorden area, Spitsbergen, Norway. In *Reports on Polar and Marine Research*; Wiencke, C., Ed.; Alfred Wegener Institute: Bremerhaven, Germany, 2004; pp 114–122.
- (26) Garcia-Plazaola, J. I.; Hernandez, A.; Becerril, J. M. Antioxidant and pigment composition during autumnal leaf senescence in woody deciduous species differing in their ecological traits. *Plant Biol.* **2003**, *5*, 557–566.
- (27) Hormaetxe, K.; Esteban, R.; Becerril, J. M.; Garcia-Plazaola, J. I. Dynamics of the alpha-tocopherol pool as affected by external (environmental) and internal (leaf age) factors in *Buxus sempervirens* leaves. *Physiol. Plant.* **2005**, *125*, 333–344.
- (28) DellaPenna, D. A decade of progress in understanding vitamin E synthesis in plants. *J. Plant Physiol.* **2005**, *162*, 729–737.
- (29) Szymanska, R.; Kruk, J. Tocopherol content and isomers' composition in selected plant species. *Plant Physiol. Biochem.* **2008**, *46*, 29–33.
- (30) Tlili, N.; Nasri, N.; Saadaoui, E.; Khaldi, A.; Triki, S. Carotenoid and tocopherol composition of leaves, buds, and flowers of *Capparis spinosa* grown wild in Tunisia. *J. Agric. Food Chem.* **2009**, *57*, 5381–5385.
- (31) Fremstad, E. *Vegetasjonstyper i Norge (Norwegian vegetation types)*; Norwegian Institute for Nature Research (NINA): Trondheim, Norway, 1997; p 279.
- (32) Sickel, H.; Norderhaug, A. Beiteadferd og nøkkelbiotoper - Foreløpige resultater fra "Levande stolar og kulturlandskap" (Grazing behaviour and key habitats - Preliminary results from "Living Summer Farms"). In *Grønn Forskning, Plantemøtet Østlandet 2001, Fagernes, Norway*; Skarstad, H. J., Ed.; Planteforsk (The Norwegian Crop Research Institute): Fagernes, Norway, 2001; pp 110–116.
- (33) Moen, A. *National Atlas of Norway. Vegetation*; Norwegian Mapping Authority: Hønefoss, Norway, 1999; p 200.
- (34) Falk, J.; Andersen, G.; Kernebeck, B.; Krupinska, K. Constitutive overexpression of barley 4-hydroxyphenylpyruvate dioxygenase in tobacco results in elevation of the vitamin E content in seeds but not in leaves. *FEBS Lett.* **2003**, *540*, 35–40.
- (35) Ching, L. S.; Mohamed, S. Alpha-tocopherol content in 62 edible tropical plants. *J. Agric. Food Chem.* **2001**, *49*, 3101–3105.
- (36) Mohamed, R.; Pineda, M.; Aguilar, M. Antioxidant capacity of extracts from wild and crop plants of the Mediterranean region. *J. Food Sci.* **2007**, *72*, 59–63.
- (37) Dörmann, P. Functional diversity of tocochromanols in plants. *Planta* **2007**, *225*, 269–276.

- (38) Abbasi, A. R.; Hajirezaei, M.; Hofius, D.; Sonnewald, U.; Voll, L. M. Specific roles of alpha- and gamma-tocopherol in abiotic stress responses of transgenic tobacco. *Plant Physiol.* **2007**, *143*, 1720–1738.
- (39) Porsild, A. E. Edible plants of the Arctic. *Arctic* **1953**, *6*, 15–34.
- (40) Staaland, H.; Brattbakk, I.; Ekern, K.; Kildemo, K. Chemical-composition of reindeer forage plants in Svalbard and Norway. *Holarctic Ecol.* **1983**, *6*, 109–122.
- (41) Ohlson, M.; Staaland, H. Mineral diversity in wild plants: benefits and bane for moose. *Oikos* **2001**, *94*, 442–454.
- (42) Tsochatzis, E. D.; Bladenopoulos, K.; Papageorgiou, M. Determination of tocopherol and tocotrienol content of Greek barley varieties under conventional and organic cultivation techniques using validated reverse phase high-performance liquid chromatography method. *J. Sci. Food Agric.* **2012**, *92*, 1732–1739.
- (43) Lid, J.; Lid, D. T. *Norsk Flora*, 7th ed.; Det Norske Samlaget: Oslo, Norway, 2005; p 1230.
- (44) Tejerina, D.; Garcia-Torres, S.; de Vaca, M. C.; Vazquez, F. M.; Cava, R. Acorns (*Quercus rotundifolia* Lam.) and grass as natural sources of antioxidants and fatty acids in the “montanera” feeding of Iberian pig: Intra- and inter-annual variations. *Food Chem.* **2011**, *124*, 997–1004.
- (45) Mogensen, L.; Kristensen, T.; Søegaard, K.; Krogh-Jensen, S. Cows fed vitamin rich feed produce vitamin rich milk. *ICROFS News* **2010**, 8–9.
- (46) Jensen, S. K. Absorption og omsætning af vitaminer. In *Kvægets ernæring og fysiologi. Næringsstofomsætning og fodervurdering (Bind 1)*; Hvelplund, T., Nørgaard, P., Eds.; Danmarks JordbrugsForskning: Foulum, Denmark, 2003; pp 375–388.
- (47) Vasta, V.; Luciano, G.; Dimauro, C.; Rohrlé, F.; Priolo, A.; Monahan, F. J.; Moloney, A. P. The volatile profile of longissimus dorsi muscle of heifers fed pasture, pasture silage or cereal concentrate: Implication for dietary discrimination. *Meat Sci.* **2011**, *87*, 282–289.
- (48) Kälber, T.; Meier, J. S.; Kreuzer, M.; Leiber, F. Flowering catch crops used as forage plants for dairy cows: Influence on fatty acids and tocopherols in milk. *J. Dairy Sci.* **2011**, *94*, 1477–1489.
- (49) Lynch, A.; Kerry, J. P.; Buckley, D. J.; Morrissey, P. A.; Lopez-Bote, C. Use of high pressure liquid chromatography (HPLC) for the determination of cda-tocopherol levels in forage (silage/grass) samples collected from different regions in Ireland. *Food Chem.* **2001**, *72*, 521–524.
- (50) Beeckman, A.; Vicca, J.; Van Ranst, G.; Janssens, G. P. J.; Fievez, V. Monitoring of vitamin E status of dry, early and mid-late lactating organic dairy cows fed conserved roughages during the indoor period and factors influencing forage vitamin E levels. *J. Anim. Physiol. Anim. Nutr.* **2010**, *94*, 736–746.